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THE DEVELOPMENT OF THE MICROSPORANGIA AND MICROSPORES OF HEMEROCALLIS FULVA.[1]

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(WITH PLATES VII–VIII).

ALTHOUGH *Hemerocallis fulva* has been a frequent subject of study, the results reported in reference to the phenomena of karyokinesis and spore formation do not seem to agree. In the *Cytologische Studien*,[2] this plant is described with multipolar spindles in the reproductive cells during the early division stages, no centrosomes being found in such cells. The division of the reproductive cells of the higher plants studied, according to the results of the several contributors to the *Studien*, is found to differ in both of these respects from the cells of the lower plants as well as from those of animals.

The spore-mother-cells of Hemerocallis are not very favorable for cytological study, the chromosomes being small and the spindles difficult to differentiate on account of a band of dark staining material found close around each nucleus; nor are the spindle threads so thick as those usually found. The dark band may be only an artifact, caused by the chromic acid of the killing solution. The material for this study was killed in chrom-acetic acid, and after being imbedded in paraffin was sectioned 12 and 14 μ thick. The stains used were iron-alum-hæmatoxylin;

[1] Contribution from the botanical laboratory of Ohio State University.

[2] Jahrbücher für wiss. Bot. **30**: 1–268. *pl. 18*. 1897.

Flemming's triple stain; a combination of anilin-safranin with iron-alum-hæmatoxylin; and anilin-safranin followed by picro-nigrosin. The last named gave the best results in staining the incipient spindles.

DEVELOPMENT OF THE MICROSPORANGIA.

A cross section of a very young stamen, at the point where the microsporangia are to be formed, shows merely a rectangular area which consists of epidermal and general tissue cells. Three or four hypodermal cells of each sporangium become differentiated as archesporial cells. These divide by periclinal division, giving rise to the primary sporogenous cells and the primary tapetal layer. The cells of the primary sporogenous tissue multiply rapidly, forming only sporogenous cells. The division of these cells is practically complete when the primary tapetal layer begins to divide. While the sporogenous cells are enlarging the division of the primary tapetal layer takes place, forming a wall layer and an inner layer. The inner layer divides once, forming an intermediate or middle layer, and the layer which develops into the peripheral part of the tapetum, the axial part being derived from the adjacent general tissue.

DIVISION OF THE MICROSPORE MOTHER CELL.

A typical spore mother cell when division begins (*fig. 1*) shows a large nucleus in the center, with radiations in the cytoplasm extending from the nucleus outward toward the cell wall. There is usually a layer of deeper staining cytoplasm next the nucleus, and one or more large nucleoli just within the nuclear membrane. The young spindle is bipolar from its first appearance, each pole being dome-shaped for a time (*figs. 2, 10*). The spindle encloses the nucleus through which the chromosomes are scattered, and the layer of deep staining cytoplasm having moved outward from the nucleus spindle lies wholly within it. The radiations above described appear to be of the same nature and to have the same function as those described in animal cells, such as fish eggs. If they are consumed in spindle formation,

they must be drawn entirely within the dark layer before the nuclear membrane has disappeared.

A careful study was made of preparations containing cells in various stages of division, many of which showed distinctly dome-shaped bipolar spindles just after the nuclear membrane had disappeared. Occasionally a nucleus was observed which seemed to have radiations extending outward, but these were never sufficiently distinct to be considered as forming a multipolar spindle. The spindle was not differentiated by the stains in any case until after the nuclear membrane had almost disappeared, as the dark band obscured the poles. At first these alone would be visible, since the remainder of the spindle could scarcely be distinguished from the nuclear membrane because of their close proximity; the central part of the spindle perhaps being in contact with the nuclear membrane. The spindle fibers at this stage are delicate, the spindle being in process of formation. The fact that the nuclear membrane disappears while the spindle is forming lends support to the theory that the material of the nuclear membrane is consumed in spindle formation.

As the poles separate, the spindle gradually becomes pointed and seems to grow somewhat narrower in the middle portion, so that it is not as wide as was the original nucleus (*figs. 1–7, 10–12*). The chromosomes are drawn into the equatorial plane (*figs. 8, 14*) soon after the spindle becomes elongated to definite points. Small deep-staining bodies which have the appearance of centrosomes are often found at the poles in the various stages of both the first and second divisions (*figs. 3, 7, 9, 13*).

In the second division of the spore-mother-cell the two nuclei may divide successively (*fig. 22*), but almost always simultaneously (*figs. 14, 16*), the spindles usually being parallel to one another (*fig. 15*), but occasionally obliquely placed (*fig. 16*), and often persisting even after division is complete (*fig. 17*). The phenomena of the first and second divisions are the same, except that in the latter the nuclei are smaller and the dark band is not so conspicuous.

Four microspores (*figs. 18, 19*) are usually produced from each spore-mother-cell, but occasionally five, six, and even eight are formed (*figs. 20–27*). Miss Lyon[3] also found this peculiarity in *Euphorbia corollata*, which in the case of Hemerocallis has been known for some time. The extra nuclei were first described as being produced by one or more of the four tetrad nuclei dividing by karyokinesis. Recently, however, they have been described by Juel[4] as being formed from chromosomes which became isolated in one of the divisions of the spore-mother-cell. Many tetrads having supernumerary nuclei were examined, only a few of which seemed to show definitely how the extra number of nuclei might have arisen. In all cases where the origin was indicated by spindles or otherwise they seemed to have been produced by a subsequent karyokinesis. The extra nuclei in *figs. 20* and *21* may be a result either of the indirect division of one of the cells of the tetrad, or of the threefold division of one of the nuclei at the second division. The division of one nucleus into three or more is quite common in animal cells in case of pathological tissue. Wilson,[5] in speaking of cases of pathological mitosis, says:

> The abnormal forms of mitoses are arranged by Hanseman in two general groups, as follows: (1) *asymmetrical mitoses*, in which the chromosomes are unequally distributed to the daughter cells, and (2) *multipolar mitoses*, in which the number of centrosomes is more than two, and more than one spindle is formed. . . . . Lustig and Galeotti ('93) showed that the unequal distribution of chromatin is correlated with and probably caused by a corresponding inequality in the centrosomes, which causes an asymmetrical development of the amphiaster.

The same author refers to the discovery of Galeotti that asymmetrical mitoses may be artificially produced in the epithelial cells of salamanders by treatment with various drugs. Guignard[6] finds very irregular and also multipolar spindles in the

[3] A contribution to the life history of *Euphorbia corollata*. BOT. GAZ. 25: 418–425. 1898.

[4] Jahrbücher für wiss. Bot. 30: 205–226. 1897.

[5] The cell in development and inheritance, 67–68. 1896.

[6] Ann. Sci. Nat. Bot. VIII. 6: 177–220, *pl. 9–11*. 1898.

spore-mother-cells of *Nymphæa alba* and *Limodorum abortivum*, and also in the tapetal cells of *Magnolia Yulan*. A number of spindles from cells of the above-named plants and also from the cells of *Nuphar luteum*, having well defined centrospheres and radiations around the poles, are shown.

If the fifth nucleus in *figs. 20* and *21* was formed by the division of one of the tetrad nuclei, the division was probably normal. In this case the presence of spindles suggesting tripolar mitosis would be explained by the persistence of spindle structures as in *fig. 17*. *Fig. 22* is similar to *figs. 20* and *21*, except that one of the cells resulting from the first division of the spore-mother-cell did not divide. *Fig. 23* may have arisen by any one of the processes described under *figs. 20* and *21*, but since there is no spindle connecting either of the two smaller nuclei with one of the larger, they may have arisen from a chromosome which was isolated in the first division of the spore-mother-cell, forming a nucleus and afterward dividing. In this case but one of the cells resulting from the first division could have divided, as there are only three other nuclei present. However, the three are nearly uniform in size, and all have the general appearance of tetrad nuclei, and not the appearance of a nucleus formed in the first division, as the elongated nucleus in *fig. 22*, which has the usual shape of such nuclei.

Fig. 24 shows four nuclei, two of which are dividing by karyokinesis. The appearance and position of the cell walls, as well as the unequal size of the nuclei, suggests that the two nuclei resulting from the first division of the spore-mother-cell were of unequal size. By the subsequent division of the nuclei thus formed two pairs of nuclei resulted; the larger pair of which are again dividing by karyokinesis. This figure may also be explained by supposing the two smaller nuclei to have been produced from an isolated chromosome. In this case the spore-mother-cell could have divided but once, and is now dividing a second time. One of the five nuclei in *fig. 25* is dividing. The source of this nucleus cannot be determined with certainty; but it may have been formed either by the indirect division or by the fragmentation

of one of the tetrad nuclei, more probably the smallest one. The fact that several, perhaps the normal number of chromosomes are present opposes the view that it arose from an isolated chromosome. Six nuclei are found in *fig. 26*, the origin of four being shown by spindles. The two extra nuclei are quite small, containing but little chromatin, and may have been produced by isolated chromosomes as above described. However, if they were produced in this manner the smallest of the four nuclei must have arisen by pathological mitosis by an unequal distribution of chromatin, for it is very small and contains but little chromatin. *Figs. 27* and *28* show tetrads with supernumerary cells. The cells of such tetrads are seldom of equal size, one or more of the cells usually containing a small nucleus.

THE DEVELOPMENT OF THE MALE GAMETOPHYTE.

The microspores soon after their separation and rounding off acquire the general shape and characteristic markings of the mature pollen grain, gradually becoming very much enlarged (*figs. 28, 30*). A single elongated nucleus, which seems to have a small amount of chromatin, is found in the center of the spore until growth is far advanced; after which the division into generative and tube nuclei occurs (*fig. 30*). Until recently it was thought that the tube nucleus never divides, but Chamberlain[7] found many cases of such division in *Lilium tigrinum* and *L. auratum*. Smith[8] found the same thing to be of frequent occurrence in *Eichhornia crassipes*. Hemerocallis also shows this peculiarity (*figs. 31–35*), many pollen grains having two, and a few having three, four, or even six tube nuclei. These were formed by direct division in all the cases that I observed, as were those reported by Chamberlain. *Fig. 33* shows a very irregular tube nucleus.

If the tube nucleus is the homologue of the cover cell of the antheridium of Marsilia, and represents the wall of an antheridium, it might sometimes divide through reversion to its more

[7] BOT. GAZ. **23**: 423–430. 1897.

[8] BOT. GAZ. **25**: 324–337. 1898.

primitive condition. Since extra tube nuclei are produced only by fragmentation, so far as known, such division may represent a pathological condition. It is possible that direct division in the higher plants never represents anything more.

SUMMARY.

1. Three or four hypodermal cells of each sporangium become differentiated as the archesporial cells. The wall of a sporangium consists of three layers exclusive of the epidermis. The tapetum is a physiological rather than a morphological structure, the peripheral part being organized from the wall layers and the axial part from the general tissue.

2. The spindle appears bipolar from its first appearance, being dome-shaped in the early stages. No trace of multipolar spindles was observed. The spindles often persist for a considerable time after division is complete. Bodies having the appearance of centrosomes are frequently seen at the poles.

3. The origin of the supernumerary microspores was not absolutely determined. In many cases where their origin was indicated by spindles or otherwise they appeared to arise by the indirect division of one of the tetrad nuclei.

4. The tube nucleus frequently divides by direct division, forming sometimes as many as six or eight nuclei.

In conclusion I wish to express my thanks to Dr. W. A. Kellerman and Mr. J. H. Schaffner for valuable assistance and criticism.

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EXPLANATION OF PLATES VII AND VIII.

The figures were drawn with a camera lucida and are reduced to three eighths their original size. *Fig. 32* was drawn with a Bausch and Lomb $\frac{1}{12}$ objective and a Leitz no. 2 ocular (× 1200); *figs. 3, 4, 9, 10, 13, 15, 21, 22, 23* with a Leitz $\frac{1}{16}$ objective and a Leitz no. 4 ocular (× 1600); all others with a Bausch and Lomb $\frac{1}{12}$ objective and a Zeiss no. 6 ocular (× 1600).

PLATE VII.

FIG. 1. Microspore mother-cell in very early stage of first division, showing dark band around nucleus and cytoplasmic radiations extending outward to the cell wall.

FIG. 2. Loose mother skein stage, showing a dome-shaped bipolar spindle which is situated entirely within the dark band.

FIGS. 3–5. Successive division stages, showing centrosome-like bodies at poles of dome-shaped spindles.

FIG. 6. Same as above, but having no bodies visible at the poles.

FIG. 7. Mother star stage; spindle pointed, having centrosome-like bodies, and with radiations at lower pole.

FIG. 8. Metakinesis stage; microspores separated.

FIG. 9. Near close of metakinesis; centrosome-like bodies and radiations present.

FIG. 10. Early stage of second division, showing dome-shaped spindle with centrosome-like bodies; dark band not so prominent as in first division.

FIG. 11. Showing one cell in a later stage of division than is the other.

FIGS. 12–14. Successive division stages.

FIG. 15. Loose daughter skein stage, showing an isolated chromosome; spindles parallel.

FIG. 16. Same as above, showing spindles lying obliquely to one another.

FIG. 17. Tetrad having nuclei variable in size and showing persisting spindles.

FIGS. 18–19. Later tetrad stages.

PLATE VIII.

FIGS. 20–21. Mother cells with five nuclei, three of which are connected by spindles, showing their common origin.

FIG. 22. Same as above, except that one nucleus did not divide after first division of spore-mother-cell.

FIG. 23. Five nuclei in mother-cell, two pairs of which are connected by spindles.

FIG. 24. Four nuclei in tetrad, two of which are dividing by karyokinesis.

FIG. 25. Mother-cell with five nuclei; one is dividing by karyokinesis.

FIG. 26. Mother-cell with six nuclei; the origin of two not shown.

FIGS. 27–28. Five- and six-celled mother cells.

FIG. 29. Microspore soon after separation.

FIG. 30. Normal mature pollen grain having one generative and one tube nucleus.

FIG. 31. Tube nucleus of pollen grain dividing by direct division.

FIGS. 32, 34. Pollen grains with one generative and two tube nuclei.

FIG. 33. Pollen grain with a very irregular tube nucleus.

FIG. 35. Pollen grain with three tube nuclei; generative nucleus not shown.

FIG. 36. Six tube nuclei in pollen grain.